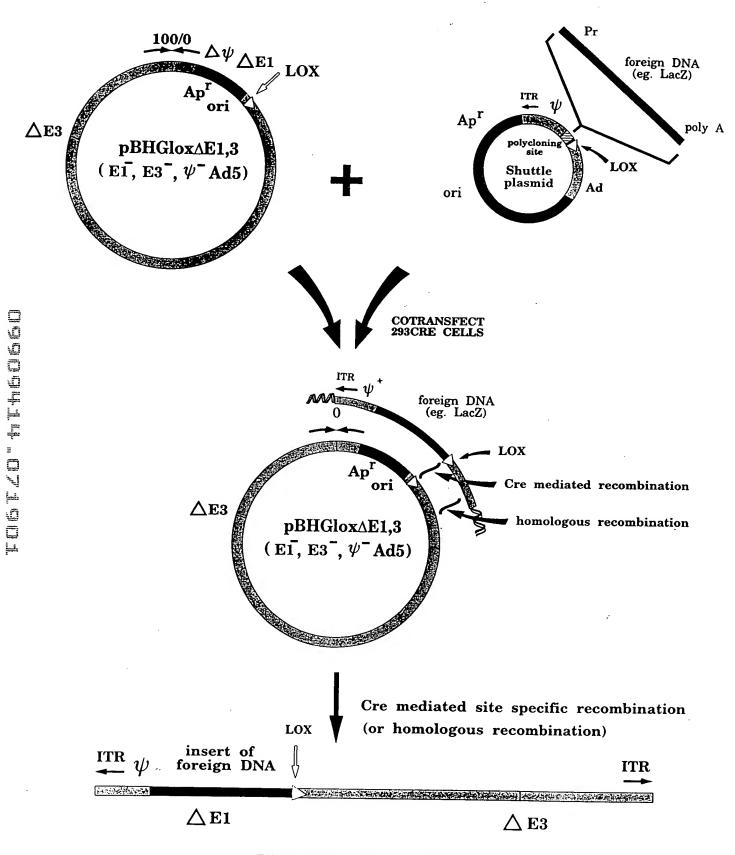


# Cotransfective of 293Cre cells with perGlox AE1,3 and a "lox" shuttle plasmid for generation of Ad expression vectors



DEFECTIVE VIRAL VECTOR

# 

#### OLIGONUCLEOTIDES USED IN CLONING

#### loxP linker Sequences

SEQ ID NO: 1

(AB3233)

BamH VBgl II overhang

5' GATCCAATAACTTCGTATAGCATACATTATACGAAGTTATAAGTACTGAATTCG3' ,

\*\* 3' GTTATTGAAGCATATCGTATGTAATATGCTTCAATATTCATGACTTAAGCCTAG 5'

BamH I/Bgl II overbang

SEQ ID NO: 2 (AB3234)

#### Multiple Cloning Site Sequences

SEQ ID NO: 3 (AB14626)

Sal I overbang

**∢**−Cla I→

| ← - Smal → | ← - Bg/ II → | ← - Hind III → | ← - Smal → |
5' AATTCCCCGGGAGATCTAAGCTTGAGCTCG 3' →

3' GGGGCCCTCTAGATTCGAACTCGAGCAGCT 5'

EcoR I overheng

SEQ ID NO: 4 (AB14627)

#### loxP linker Sequences

SEQ ID NO: 5 (AB6920)

Xba I overhang

5' CTAGCAATAACTTCGTATAGCATACATTATACGAAGTTATATCGATG 3' 

\* 3' GTTATTGAAGCATATCGTATGTAATATGCTTCAATATAGCTACGATC 5'

Xba I overhang

SEQ ID NO: 6 (AB6921)

#### loxP linker Sequences

SEQ ID NO: 7 (AB14680)

Blp I overhang

5' TGACAATAACTTCGTATAGCATACATTATACGAAGTTATATCGATG 3'
3' GTTATTGAAGCATATCGTATGTAATATGCTTCAATATAGCTACACT 5'

SEQ ID NO: 8 (AB14681)

Blp I overhang

## CONSTRUCTION OF A CIRCULAR GENOMIC PLASMID FOR Ad VECTOR RESCUE USING THE Cre/ loxP SYSTEM

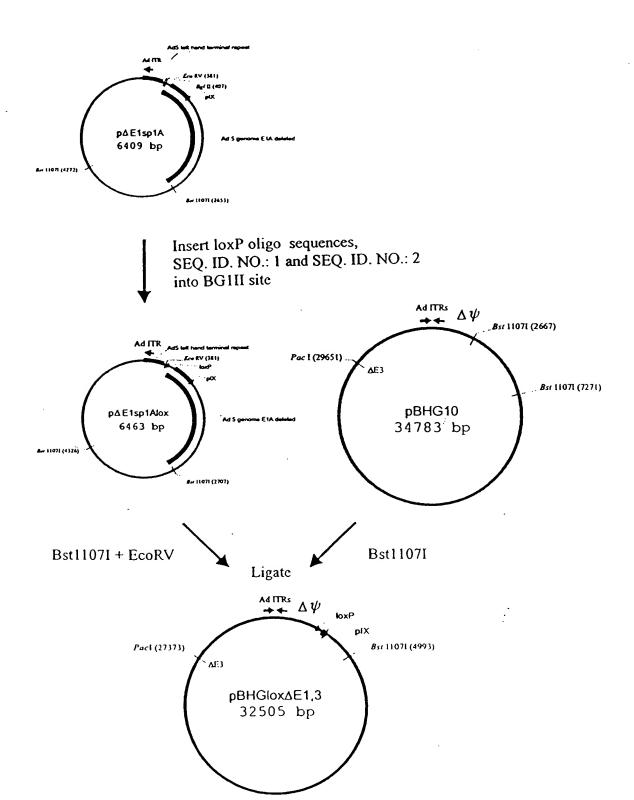
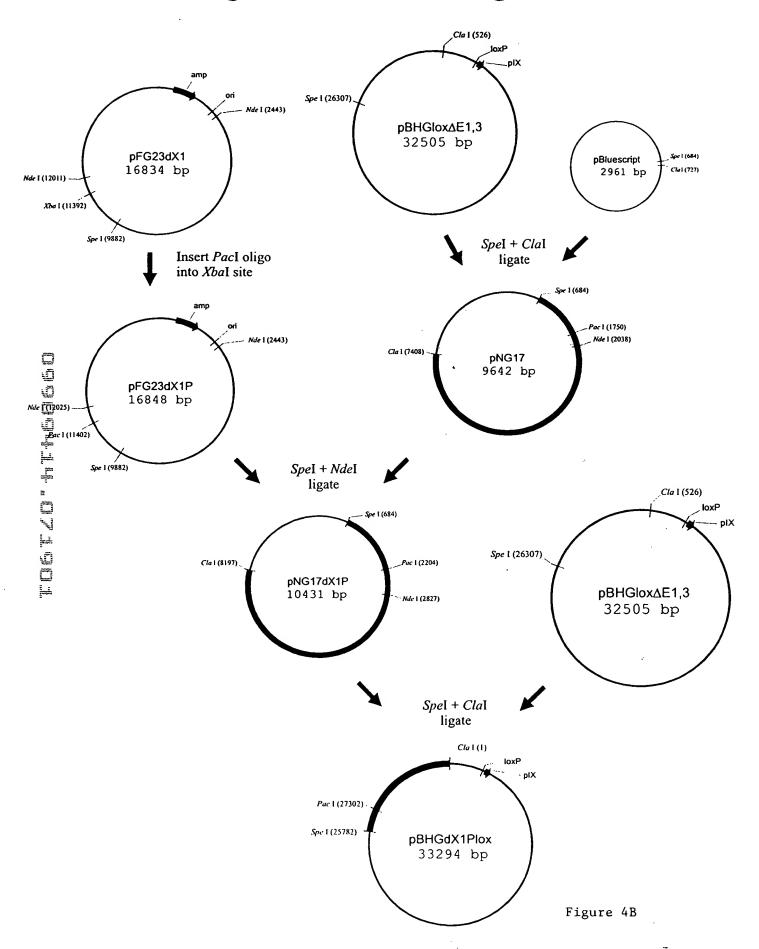
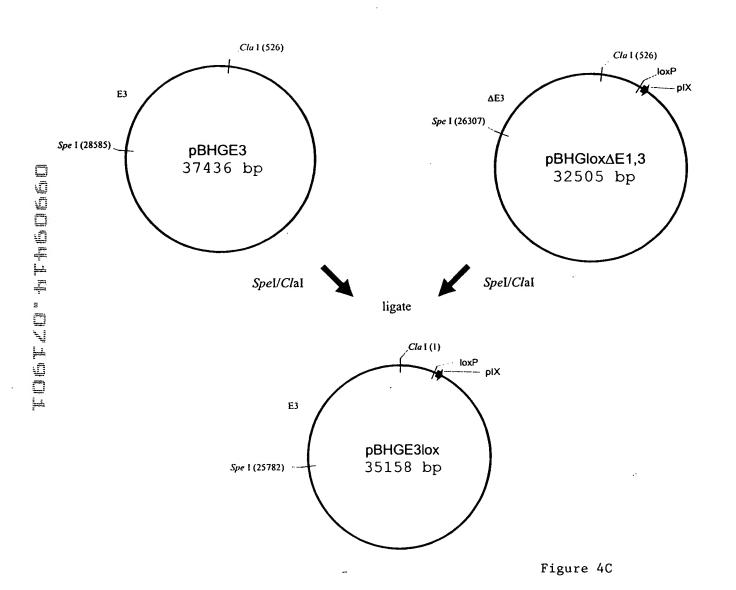


Figure 4A

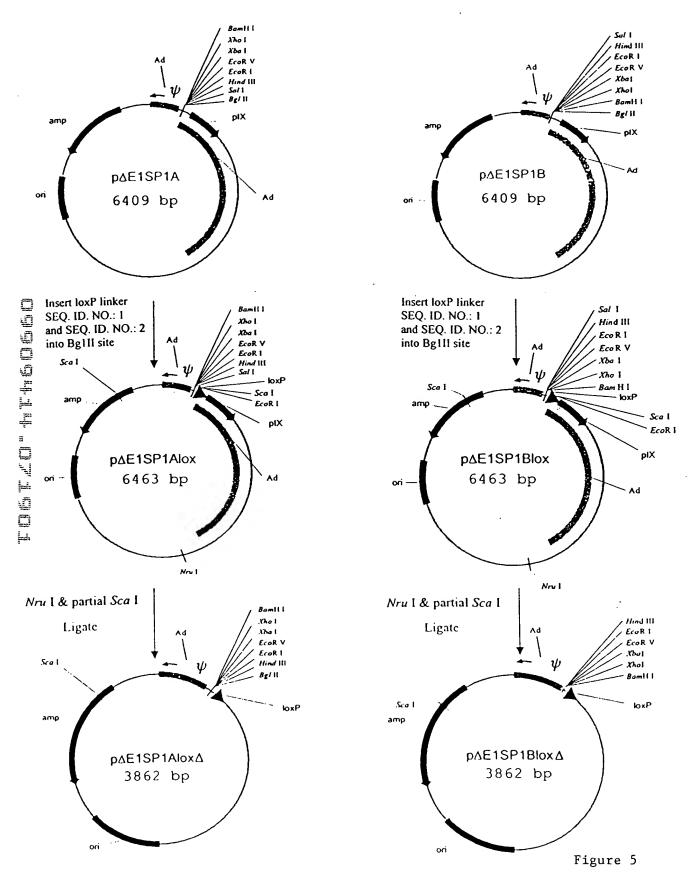
### COSTRUCTION OF pBHG Plox



## CONSTRUCTION OF pBHGE3lox



# CONSTRUCTION OF pΔE1SP1A & pΔE1SP1B loxP PLASMIDS FOR RESCUE OF FOREIGN DNA





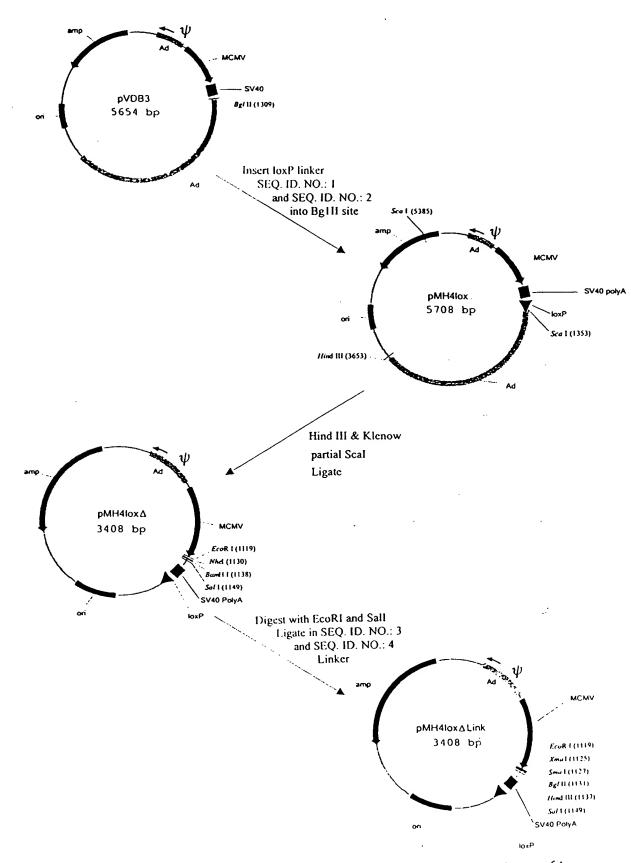
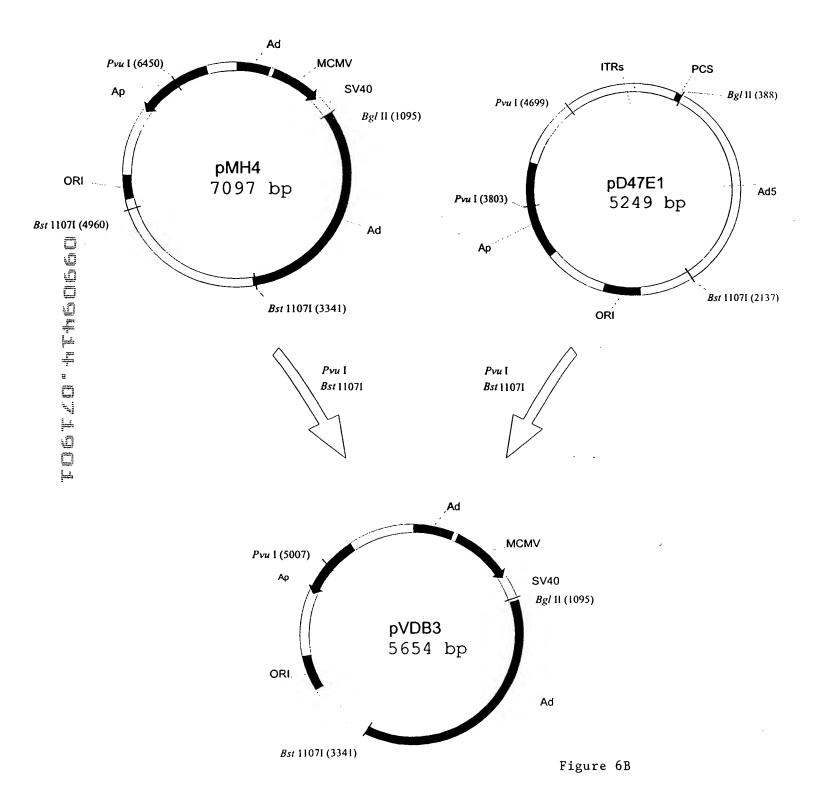
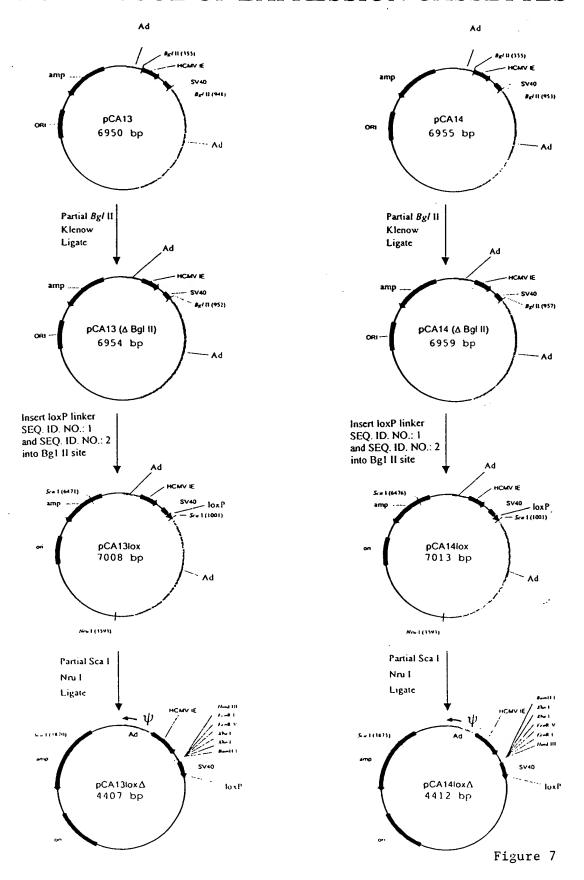


Figure 6A

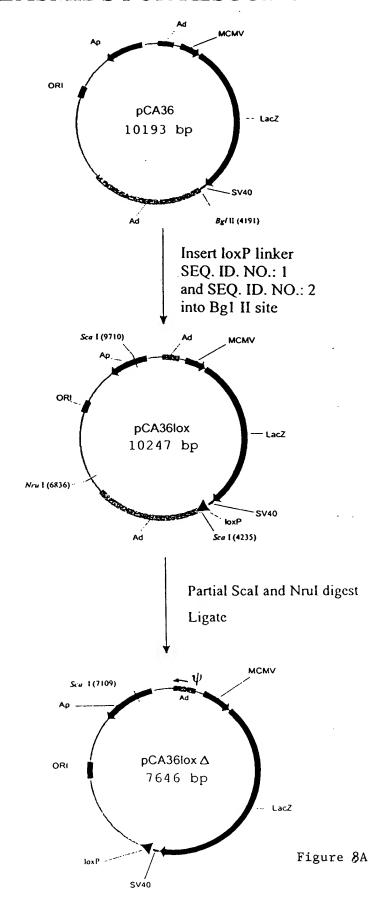
# CONSTRUCTION OF A SHUTTLE PLASMID CONTAINING A pUC DERIVED ORIGEN



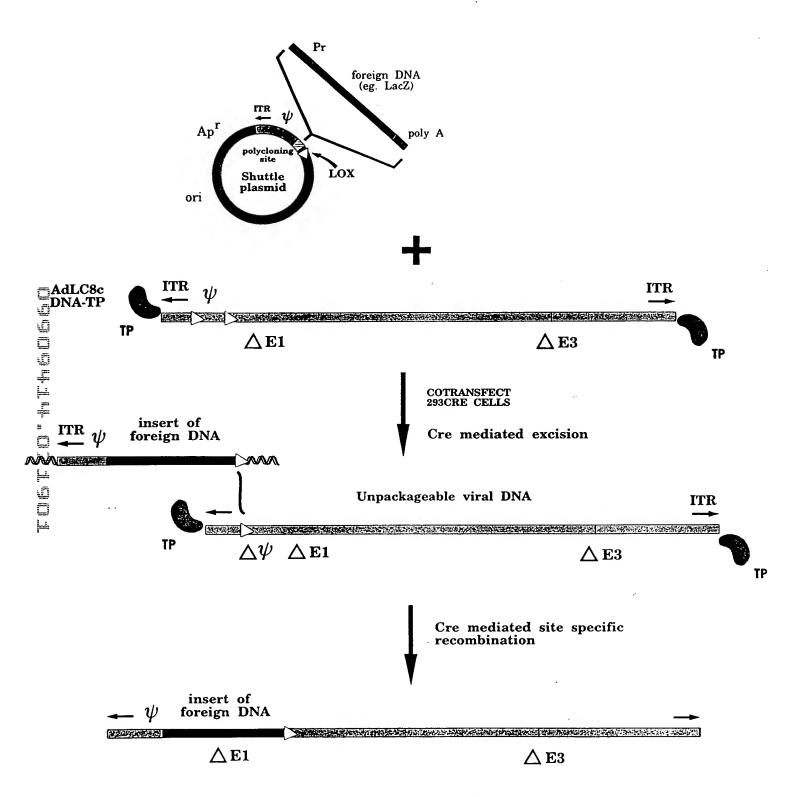
# CONSTRUCTION OF HCMV loxP PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES



# CONSTRUCTION OF pCA36LOX and pCA36LOX \( \Delta\) SHUTTLE PLASMIDS FOR RESCUE OF LACZ

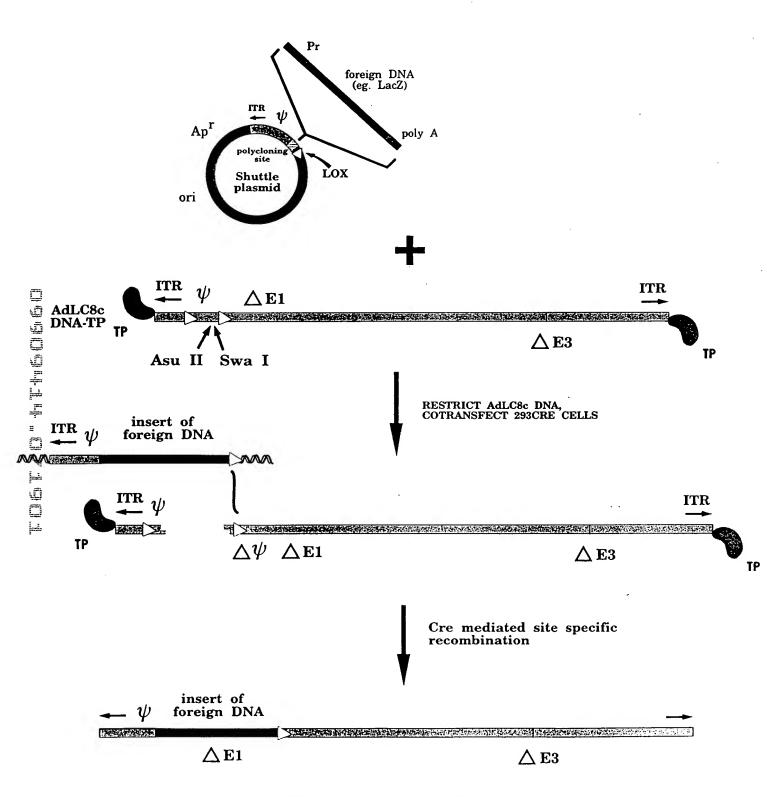


## Cotransfection of 293Cre cells with AdLC8c DNA-TP and a shuttle plasmid containing a loxP site for generation of Ad expression vectors



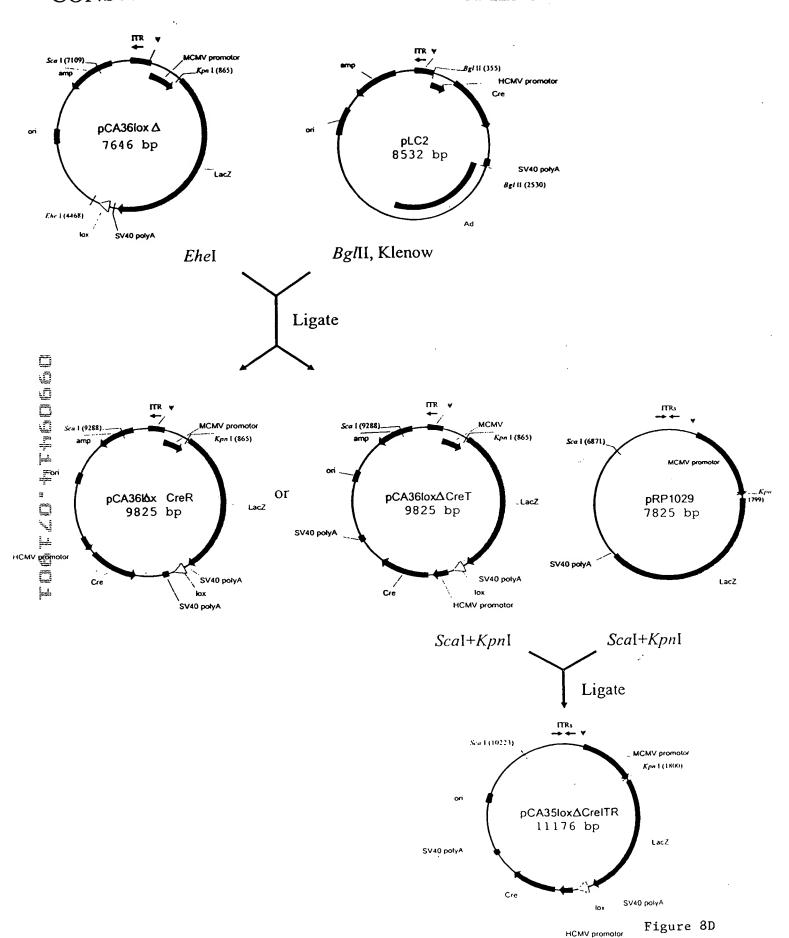
DEFECTIVE VIRAL VECTOR

## Cotransfection of 293Cre cells with restricted AdLC8c DNA-TP and loxP shuttle plasmid for generation of Ad expression vectors

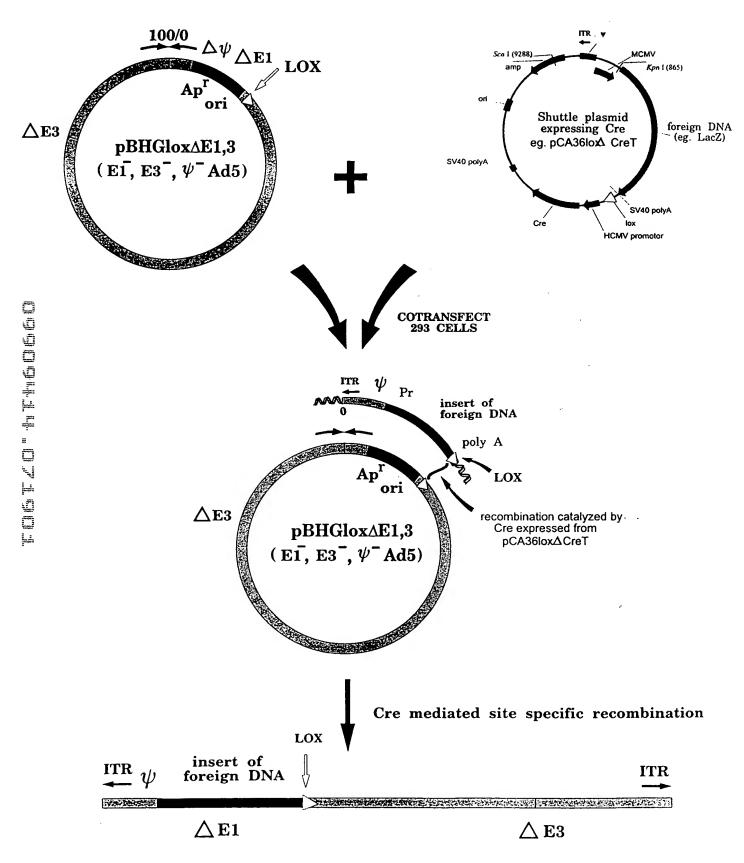


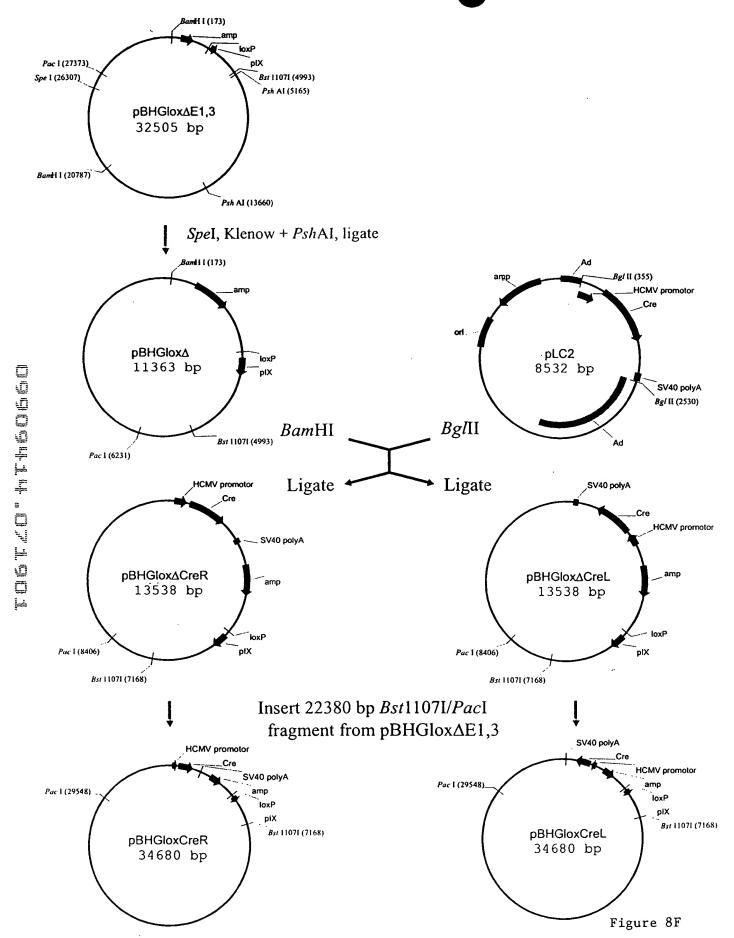
DEFECTIVE VIRAL VECTOR

## CONSTRUCTION OF SHUTTLE PLASIONS EXPRESSING Cre

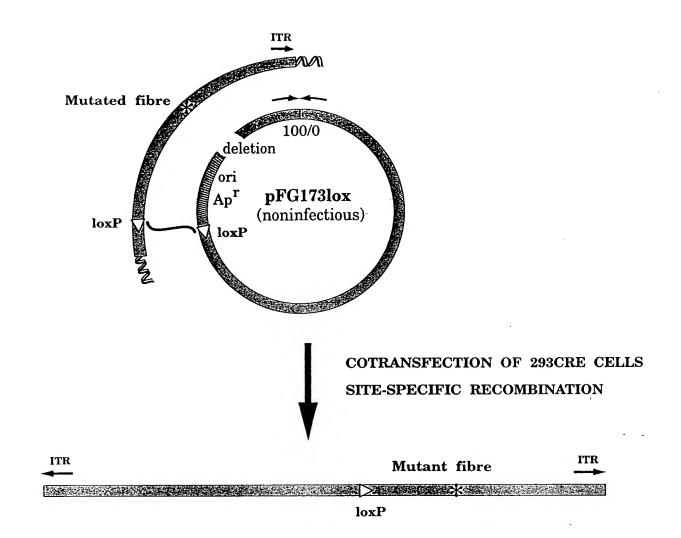


## Cotransfection of the cells with pBHGloxAE and a 'Lox' shuttle plasmid expressing Cre for generation of Ad expression vectors



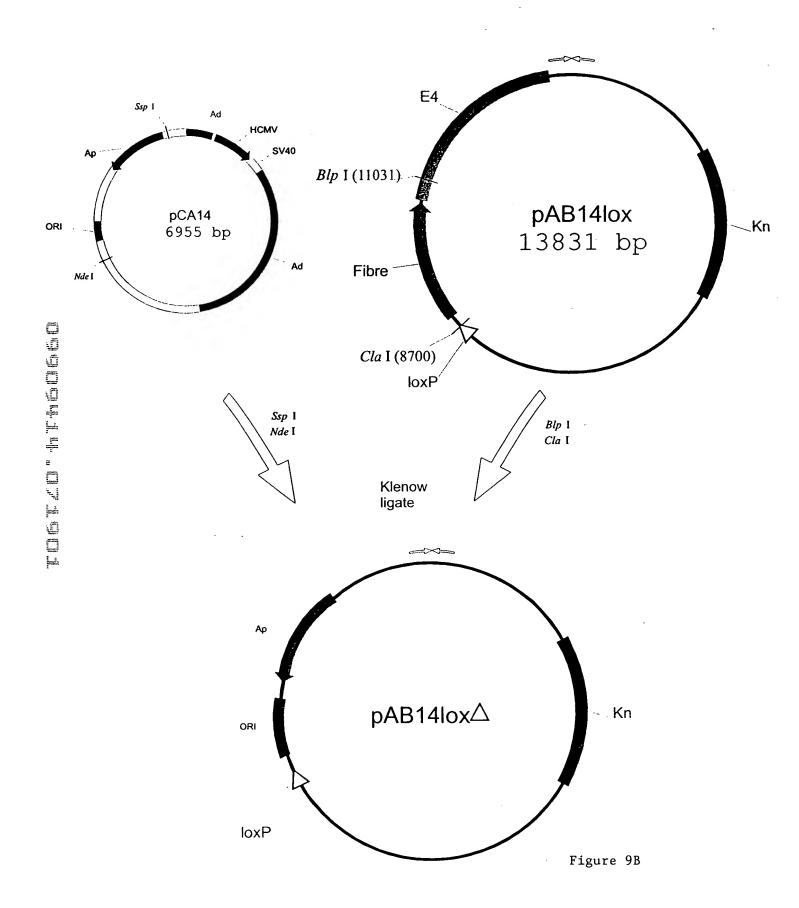


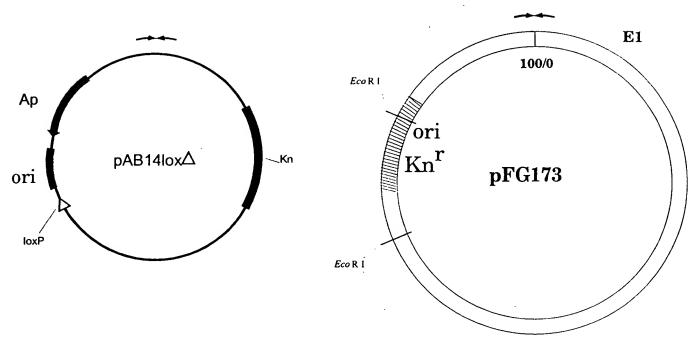
# RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION



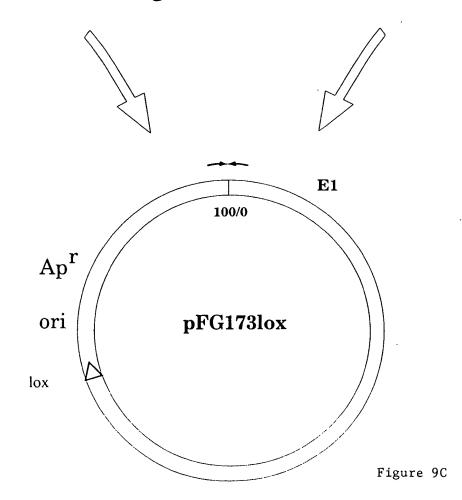
NONDEFECTIVE (E1<sup>+</sup>) VIRUS WITH MUTATED FIBRE GENE

## CONSTRUCTION OF pAB14lox $\triangle$

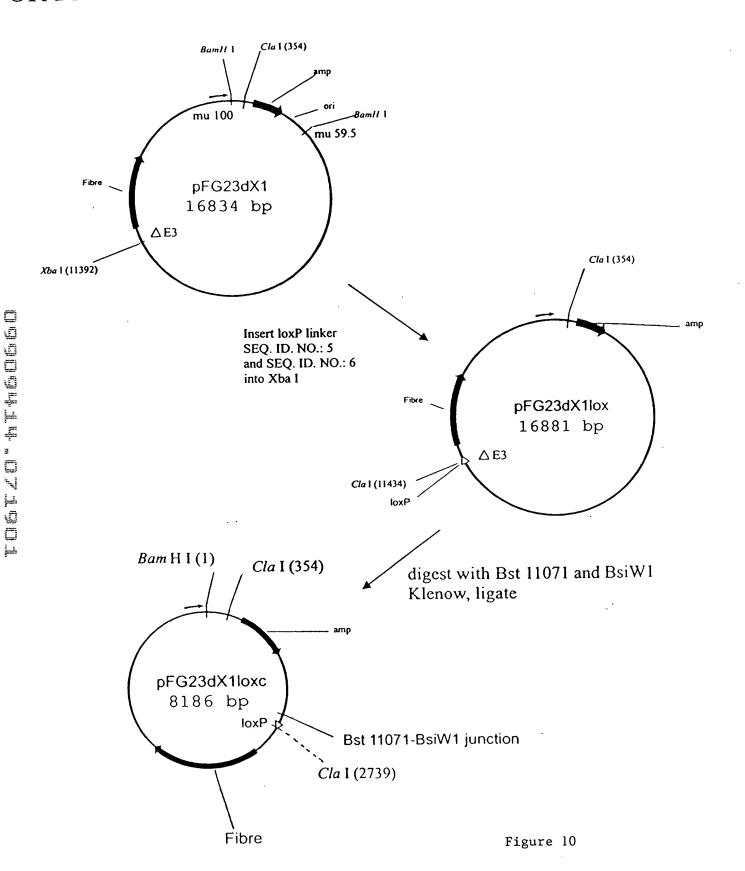




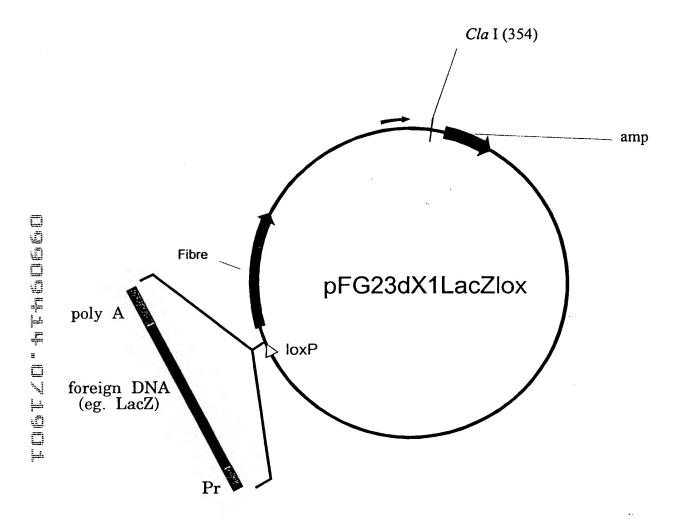
Restriction, transformation of E. coli, homologous recombination



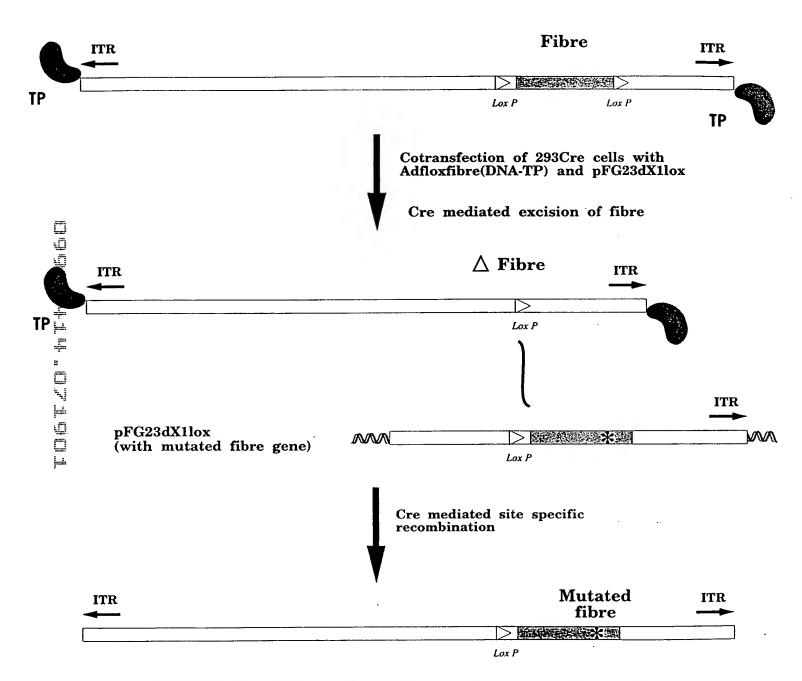
# CONSTRUCTION OF pFG23dX1lox AND pFG23dX1loxc FOR RESCUE OF MUTANT FIBRE INTO AD VIRUS



#### A PLASMID FOR RESCUE OF A FOREIGN DNA INTO AD VIRUS

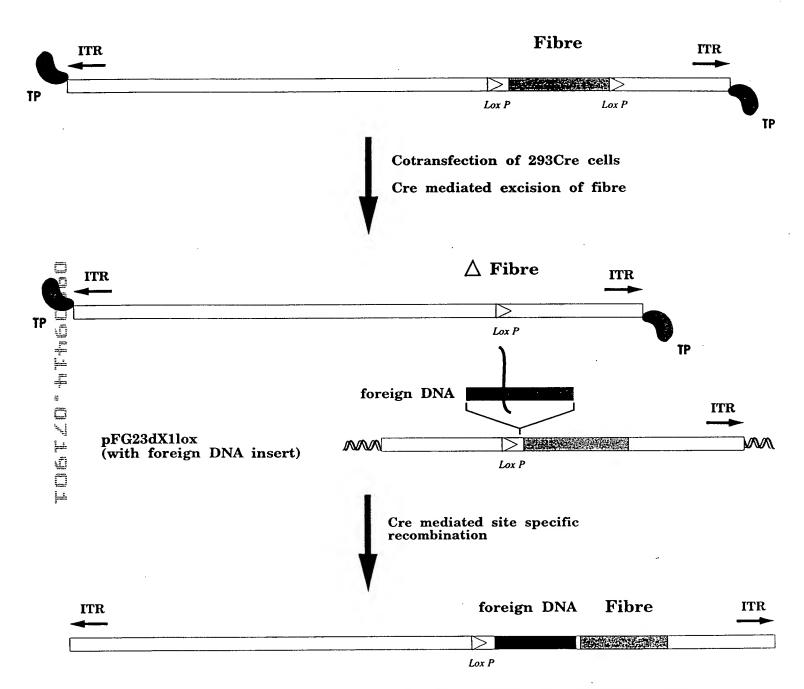


#### Isolation of a virus containing a mutant fibre gene by Cre-lox recombination using DNA-TP and cotransfection



RECOMBINANT VIRUS CONTAINING A MUTATED FIBRE GENE

## Isolation of a virus containing a foreign DNA insert upstream of the fibre gene by Cre-lox recombination



RECOMBINANT VIRUS CONTAINING AN INSERT OF FOREIGN DNA UPSTREAM OF THE FIBRE GENE

# CONSTRUCTION OF pAB14FL0X FOR ISOLATION OF AN AD VIRUS WITH A FLOXED FIBRE GENE

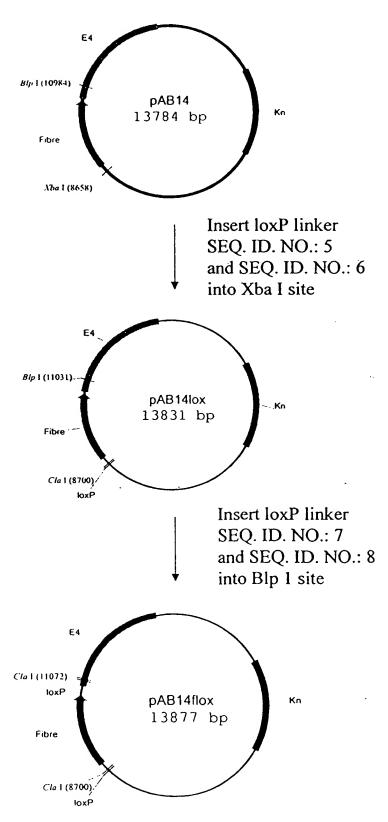
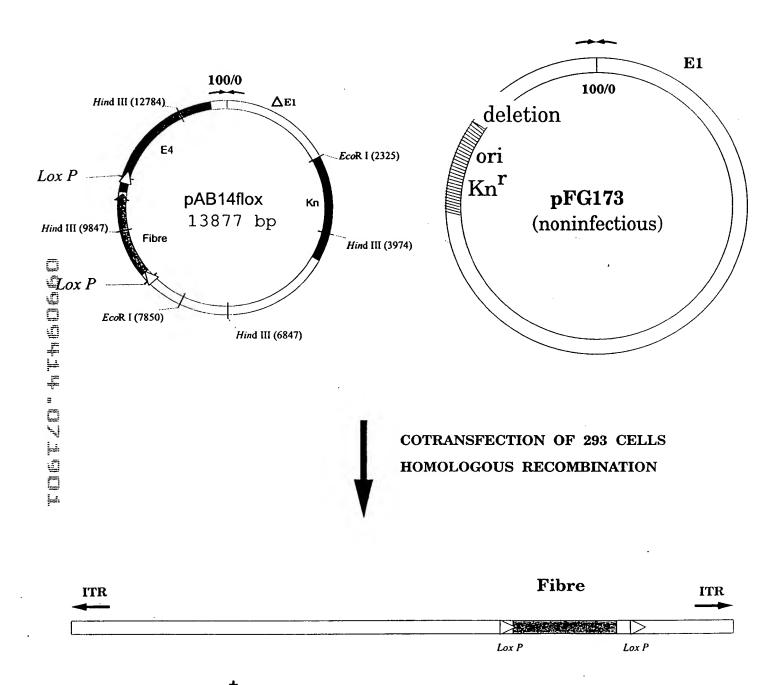


Figure 14

# Isolation of a virus containing a fibre gene with flanking lox P sites.



NONDEFECTIVE (E1 ) VIRUS (ADFLOXFIBRE) CONTAINING A FLOXED FIBRE GENE